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Ultraviolet Sensitivity Gene of *Escherichia coli* B

JOHN DONCH AND JOSEPH GREENBERG
Palo Alto Medical Research Foundation, Palo Alto, California 94301

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The ultraviolet sensitivity gene of *Escherichia coli* B was introduced into a K-12 recipient by transduction with phage P1. The *uvs* gene of *E. coli* B is cotransducible with the *proC* locus of K-12, is closely linked to *tsx*, is not linked to *lacZ*, and only rarely to *purE*. The transductants are mucoid, filamentous on irradiation, and show plating-medium response. The order of markers is *lacZ proC tsx uvs purE*.

Escherichia coli B is sensitive to ultraviolet irradiation (UV), forms filaments upon exposure to low doses of UV (6–8), is less sensitive to UV when plated on minimal medium than when plated on complex medium (plating-medium recovery, PMR; 12), and is less sensitive to UV when incubated after irradiation at 42 C than after irradiation at 37 C (heat recovery; 2). In addition, strain B is not mucoid even under conditions, such as growth on minimal medium at low (30 C) temperatures, which provide for the maximal expression of the mucoid (Mu) phenotype (9, 11). [Most strains of *E. coli* are mucoid, i.e., colonies are large, spreading, and watery when grown for 3 or more days on minimal medium at 30 C. At higher temperatures and on complex media, they tend to form glistening colonies, which, however, are not spreading or watery. As used in this report, mucoid (Mu) strains produce large, spreading watery colonies even at 37 C on minimal medium (Fig. 1); some mucoid strains form such colonies on complex media. Strain B does not form mucoid colonies under any conditions.]

The locus of the gene (*uv*) responsible for the above characteristics was mapped by Greenberg (4) in the *lac tsx* region. [We shall refer to the gene for UV sensitivity in strain B as the *uv* gene. All other gene and phenotype designations, unless otherwise described, conform to the suggestions of Demerec et al. (3).] By using crossover frequencies, Greenberg determined the order of genes to be *lac tsx uv*. Howard-Flanders et al. (7) isolated mutants of the K-12 derivative AB1157 which had UV survival curves similar to that of strain B, became filamentous on UV irradiation, and were Mu (even at 37 C, unlike parental AB1157). These were considered to be mutants in a gene, *lon*, which was mapped by sexual recombination in the region of *lac* and *tsx*. Markovitz (9) also isolated mucoid, UV-sensitive mutants of strain K-12. He found that the gene, R_1, now *capR* (10), regulating the mucoid property was not cotransducible with *lac*; however, it was contransducible with *proC*, which was cotransducible with *lac*.

There may be at least two reasons why strain B is not mucoid: (i) its *uv* gene is not the same as the *lon* gene, or the *capR* gene, or (ii) it is the same but there is another mutation which inhibits mucous production as suggested by H. I. Adler and A. A. Hardigree (*private communication*). In this report we shall show that, when the *uv* locus of *E. coli* B is transferred to the nonmucoid, UV-resistant, *proC* strain χ478 by cotransduction with $proC^+$ by phage P1, the transductants produce filaments upon exposure to low doses of UV, show plating-medium recovery, and are mucoid. The order of markers in the region is *lac proC tsx uv purE*. The *uv* locus may be the same as *lon*.

MATERIALS AND METHODS

Bacteria. Bacterial strains used are described in Table 1. The survival after UV irradiation of strain B and χ478 is shown in Fig. 3.

Phage. P1kc was supplied to us by Charles Yanofsky, and from this we isolated a virulent mutant, P1 *vir*, which overcomes immunity of P1 lysogenic strains and produces large, clear plaques.

P1 *vir* has a low efficiency of plating on strain B. Therefore, it was grown by the overlay method on strain B, and a single plaque was picked and replated on strain B. After two cycles of plating on strain B, a high-titer preparation for strain B was obtained, and this grew efficiently on K-12 strains. This strain was used in all experiments, so we shall refer to it as P1. P1 was grown on donor strains for at least two cycles and harvested as described by Adams (1).

Transductions. Transductions were performed by

FIG. 1. *Transduction of proC⁺ to χ478 by P1 grown on strain B, plated on minimal glucose medium, and incubated at 37 C for 3 days. Note mucoid colonies.*

using bacteria grown overnight with aeration in DK broth (*see* below) supplemented with 2.5×10^{-3} M $CaCl_2$, diluted 1:10 into fresh broth of the same composition, and incubated for 2 hr (about 8×10^8 cells/ml) with aeration. All incubations were at 37 C. P1 was then added to give a multiplicity of infection of 0.05 to 0.1. Adsorption was allowed to proceed without shaking for 30 min, after which the adsorption mixture was centrifuged at approximately 3,300 × *g* for 15 min. The supernatant fluid was decanted and used to determine the percentage of phage adsorbed. The pellet was resuspended in water and appropriately diluted; 0.1-ml amounts were then spread onto selective media.

As controls, P1 lysates were spotted on DK agar to test for bacterial sterility; to test for reversions, a sample of recipient cells was treated as were the experimental cells but without P1. Bacterial survival was determined by plating on complete medium appropriate dilutions of cells before adding P1 and also at the end of the adsorption period. All selection plates were incubated from 2 to 5 days. Transductants to prototrophy or to Lac⁺ were purified at least once on selective medium.

Media. The minimal medium used for selection in transduction experiments was Davis Minimal (DM) Broth (Difco) to which Noble Agar (Difco) was added at a final concentration of 2%, glucose (or lactose in Lac⁺ selections) at a concentration of 0.5%, and streptomycin (to prevent contamination) at 200 μg/ml; amino acids were used at a concentration of 50 μg/ml, and vitamin B_1 at a concentration of 0.17 μg/ml. The purine requirement was satisfied interchangeably by adenine, adenosine, guanine, or guanosine at a concentration of 5 μg/ml.

Complete (DK) broth consisted of tryptone, 5 g; NaCl, 5 g; and glucose, 1 g per liter of deionized water. Viable counts, survival curves, and filament formation studies were done on this medium, without glucose, solidified with 1.5% Bacto Agar (Difco).

Filament formation. Filaments were induced by first growing cells overnight with shaking at 37 C in DM Broth plus supplements required by the strain. After overnight growth, the cultures were diluted in supplemented DM Broth (1:50 or 1:100, depending upon growth rate), and incubated with shaking until a titer of approximately 10^8 to 4×10^8 cells/ml was reached; 1.0-ml amounts were placed in 60-mm petri plates and exposed to 75 ergs/mm² of UV light from a Westinghouse germicidal lamp producing 15.4 ergs per mm² per sec at a distance of 51.5 cm. Appropriate dilutions were spotted onto ringed slides (Perma-Slides, Progressive Laboratory Specialties, Inc.) set in a 150-mm petri plate. Slides were held above the bottom of the petri plate by applicator sticks. A moist filter paper on the bottom of the petri plate prevented drying of the spots. The entire assembly was covered by the petri cover and incubated for 2 hr. The slides were then examined under 100 × magnification for the presence of elongated cells. Under these conditions, filamentous cells were at least four to five times normal cell length.

Alternatively, diluted, irradiated (75 ergs/mm²)

TABLE 1. *Characteristics of bacterial strains used*[a]

Strain	Relevant markers					Fil	Mu	Source
	lacZ	*proC*	*purE*	*tsx*	*str*			
B (CSH)	+	+	+	+	+	+	−	R. Hill
B251	+	+	+	+	+	+	−	W. Arber
χ478	−	−	−	−	−	−	−	R. Curtiss III
PAM 42	−	+	−	+	−	+	+	Transduction of χ478 by P1·B251
PAM 43	−	−	+	−	−	+	+	Transduction of χ478 by P1·B251
W1485	+	+	+	+	+	−	−	C. Yanofsky

[a] Abbreviations are as recommended by Demerec et al. (3). Fil⁺ means the strain forms long filaments after UV irradiation; Mu⁺ means that colonies produce large amounts of mucoid material at 37 C on minimal medium. For *tsx* and *str*, a plus sign indicates that the strain is susceptible, and a minus sign indicates resistance.

cells were spotted onto DK agar, incubated for 3 hr, and then examined under 100 × magnification. Under these conditions, filamentous cells were from 10 to 50 times normal cell length.

Initial classification of filamentous strains produced by transduction was done coincidentally with testing for UV sensitivity. The rapid streak method described in Greenberg (4) was used. UV-irradiated streak plates (1,078 ergs/mm²) were incubated overnight, and filament formation was determined by microscopic examination of areas within the streaks. Under these conditions, filamentous cells were three to four times normal cell length.

UV survival curves. UV survival curves were obtained by the methods described by Greenberg (5), with the exception that log-phase cells were used instead of stationary cultures.

PMR. PMR was tested exactly as described for UV survival curves except that irradiation was performed on cells plated on DM agar and compared with survival on DK agar.

All operations involving filament formation, survival curves, and PMR were performed in subdued light to avoid photoreactivation.

RESULTS AND DISCUSSION

In preliminary experiments, when P1 was used to transduce *proC*$^+$ from strain B to χ478, a minority of the transductants were Mu. Several of the colonies were reisolated and tested for their UV sensitivity and for UV-induced filaments. They were found to have UV sensitivity curves similar to that of the donor strain B, and to become filamentous on UV irradiation. In being UV-sensitive, filament-inducible, and mucoid, they resembled AB1899, the *lon* mutant described by Howard-Flanders, Simson, and Theriot (7). Two hundred Mu transductants were eventually collected and purified, and all were found to be similar to strain B in sensitivity to UV by the rapid streak test. None of 800 nonmucoid transductants tested was UV-sensitive. It is evident that UV sensitivity and mucoidy are pleitropic properties of one gene.

Definitive experiments were performed in which not only *proC*$^+$, but also *lacZ*$^+$ and *purE*$^+$, were transduced from strain B to strain χ478. These transductants were examined for unselected markers (Table 2).

When *lacZ*$^+$ transductants were selected, all were *proC*$^+$, whereas selection for *proC*$^+$ yielded only 7% *lacZ*$^+$ transductants. This lack of reciprocity was noted by Schwartz (13) and seems to be a property of all *proC* strains (R. Curtiss, III, *personal communication*). Of the *lacZ*$^+$ transductants, 7% were T6-sensitive. None of the 4,230 *lacZ*$^+$ transductants observed on the primary selection plate was mucoid. In addition, when 500 *lacZ*$^+$ transductants were restreaked on the primary selection medium, no segregation of nonmucoid to mucoid was seen and all were found to be UV-resistant.

When *proC*$^+$ transductants were selected, 7% of the clones were mucoid. Of 200 mucoid transductants analyzed, only 1 had received the *lacZ*$^+$ gene. All mucoid transductants were UV-sensitive and none of the nonmucoid transductants was UV-sensitive. When nonmucoid *proC*$^+$ transductants were analyzed, 7% had received the *lacZ*$^+$ locus and 13% received the *tsx* sensitivity gene of *E. coli* B. Further *lacZ*$^+$ and *tsx* sensitivity segregated independently.

Three hundred nonmucoid *proC*$^+$ transductants were purified, including the 200 in the above analysis, to test whether nonmucoid clones were heterogenotes. Such a state would require an adjustment of the actual frequency of transduction of Mu, since that stated in Table 2 reflects an integrated frequency. The actual frequency of transduction could be higher, if there were a delayed integration due to the formation of heterogenotes in which mucoidy was not expressed. Only two heterogenotes were obtained. Both were sensitive to lysis by phage T6. They segregated large numbers of mucoid colonies which were UV-sensitive and T6-sensitive. Thus, the actual frequency of cotransduction of mucoidy as given in Table 2 is not in error by more

TABLE 2. *Linkage relationships among lacZ*$^+$, *proC*$^+$, *and purE*$^+$ *and genes controlling UV sensitivity and mucoidy*

Selected marker[a]	No. examined	Frequency of unselected donor markers (%)[b]					
		lacZ$^+$	*proC*$^+$	*tsx*$^+$	*uv*	Mu	*purE*$^+$
lacZ$^+$	500	100	100	7	0	0	0
proC$^+$	3,622	7	100	21	7	7	0
Nonmucoid	200	7	100	13	0	0	0
Mucoid	200	0.5	100	95	100	100	0
purE$^+$	6,234	0	0	0	0.1	0.1	100

[a] P1 donor was B251 and recipient was χ478.

[b] Six transductants were UV-sensitive and mucoid; *uv* means sensitive to ultraviolet radiation.

than 1%. A more important conclusion is that the gene controlling mucoidy behaves as though it were recessive to the wild-type, nonmucoid allele. Since UV sensitivity is pleiotropic with mucoidy, it too behaves as a recessive allele.

Table 2 shows that *purE*$^+$ transductants rarely received the *uv* locus of strain B and none of 6,234 transductants received the markers *proC*$^+$, *tsx*$^+$, or *lacZ*$^+$. These results are compatible with the order of markers shown in Fig. 2. The order of some of the genes shown in Fig. 2 was anticipated by Greenberg (4) from data obtained by conjugation of *E. coli* B recipients with *E. coli* K-12 Hfr donors. The data presented here more precisely order the *lacZ-purE* region.

However, it is obvious that the cotransductional frequencies require cautious interpretation of linkages. For one thing, the lack of reciprocity in a transduction of *lacZ* and *proC* is not understood. For another, the cotransductional frequencies of *lacZ* and *tsx*, and *proC* and *tsx*, are more than additive. Furthermore, one would predict from the linkages of *lac* and *tsx*, together with the linkage of *tsx* and *uv*, that some of the 500 *lacZ*$^+$ transductants in Table 2 would also carry the *uv* gene of strain B, but none did. Finally, the linkage of *proC* and the *capR* gene are cotransducible at a frequency of 20%, according to Markovitz (9, 11). This might mean that the *capR* gene and the *uv* gene of strain B are not the same. However, in experiments with P1·K, *lacZ*$^+$ and T6 sensitivity were cotransduced with *proC*$^+$ at frequencies of 22 and 37%, respectively. In addition, when *lacZ*$^+$ was selected, *tsx*$^+$ was cotransduced at a frequency of 20%. These frequencies are somewhat higher than those in Table 2 and point out the dependence of cotransductional frequencies on properties of the transducing particles, perhaps as determined by the properties of the donor strain of bacteria.

Some other properties of UV-sensitive mucoid transductants were examined. All were found to form long filaments after irradiation with UV. In addition, all of the UV-sensitive transductants were subject to PMR. Data on two of these are shown in Fig. 3. It is apparent that the mucoid transductants of χ478 were more sensitive to UV than was χ478, their survival curves resembling that of parental strain B. Furthermore, they were more sensitive to UV when plated on complex DK medium than on DM medium.

It is clear that, when the *uv* gene of strain B is transduced into a K-12 derivative, mucoidy is expressed. Furthermore, the *uv* gene of strain B and the *capR* gene are linked to *proC*, as is the *lon* gene (Donch and Greenberg, *in preparation*). However, the data do not exclude the possibility that there may be more than one gene linked to *proC* concerned with UV sensitivity, though it is also possible that the *lon* cistron, the *uv* cistron of strain B, and the *capR* cistron are the same. Strain B does not express the mucoid phenotype, probably because, as suggested by Adler and Hardigree (*private communication*), it is also mutant at another gene concerned with some step in the synthesis of mucoid polysaccharide. Nonmucoid derivatives of the *lon* mutant AB1899 were isolated by Adler and Hardigree and found

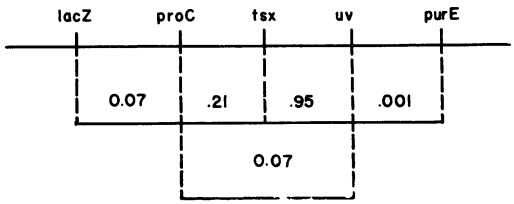

FIG. 2. *Genetic map of the proC region of the Escherichia coli chromosome. The distances are given in terms of cotransduction frequencies, determined with phage P1. The donor was either B (CSH) or B251. The recipient was χ478.*

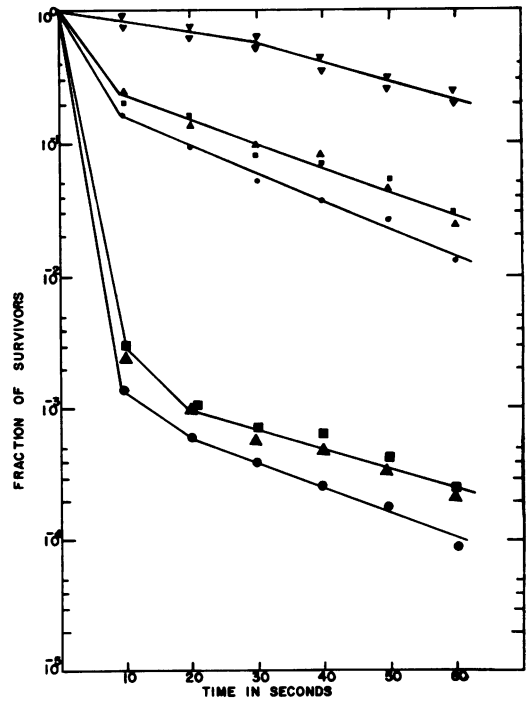

FIG. 3. *Survival after UV irradiation of Escherichia coli χ478 (inverted triangles) and B(○, ●), and of two mucoid, proC*$^+$ *transductants of χ478 by P1·B, PAM 43(□, ■) and PAM 42(△, ▲). Open symbols, plated on minimal medium; closed symbols, plated on complex medium.*

to retain all of the properties of *lon* mutants except mucoidy. The second mutation which turned off the synthesis of mucoid polysaccharides was linked to *his*. We have no information as to whether there is in strain B a mutant gene, linked to *his*, which interferes with the synthesis of the polysaccharide. Since the synthesis of mucoid polysaccharide is a multistep process (9), it is conceivable that any one of a number of steps could be turned off by mutation.

Other moot points now under investigation are the relationships among filament formation after irradiation, UV sensitivity, and the regulation of mucous production, all of which are controlled by one cistron. Also, it has not yet been clearly established that mutations in strain B leading to UV resistance, loss of UV-induced filament formation, and PMR are the result of back mutations at the *uv* locus.

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